Single Doses of Antibody Drug Conjugates (ADCs) Targeted to CD117 or CD45 Have Potent In Vivo Anti-Leukemia Activity and Survival Benefit in Patient Derived AML Models

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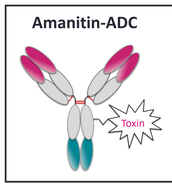
BACKGROUND

Allogeneic bone marrow transplant (BMT) is a potentially curative approach in patients with refractory or high risk hematologic malignancies, such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Prior to transplant, patients are prepared with non-specific, high dose chemotherapy alone or in combination with total body irradiation, which are associated with early and late morbidities, including organ toxicities, infertility, secondary malignancies, and substantial risk of mortality. As a result, many eligible patients do not consider transplant and of those transplanted, 2/3 can only tolerate reduced intensity conditioning, which is associated with increased relapse rates (Scott et al. Journal of Clinical Oncology 2017, 1154-1161). Thus, safer and more effective conditioning agents with improved disease control are urgently needed. To meet this need, we developed two novel antibody drug conjugates (ADCs) conjugated to amanitin (AM) targeting CD117 (C-KIT, Pearse 2018), which is expressed on hematopoietic stem and progenitor cells and AML and MDS cells in ~80% of patients (Gao et al. PLOS One. 2015), and CD45 (Palchaudhuri 2018) which is expressed on all lympho-hematopoietic cells and nearly all hematologic malignancies except multiple myeloma. The aim of the project was to design a non-genotoxic agent with the dual benefit of depleting primary human hematopoietic stem progenitor cells (HSPCs) while reducing disease burden in leukemia models.

METHODS

ADCs were tested in xenograft murine models inoculated with human leukemia cells from immortalized cell lines [Kasumi-1, a CD117 expressing leukemia cell line ($5x10^6$ cells/mouse), and REH-Luc, a CD45 expressing ALL cell line tagged with luciferase ($5x10^6$ cells/mouse), and three patient-derived xenografts (PDX) developed from FLT-3+NPM1+ AML samples [AML #1 (J000106132), AML #2 (J000106565), AML #3 (J000106134)] with varying growth kinetics (median survival of vehicle treated groups was 43, 63, 82 days post inoculation) that express both CD117 and CD45 (Jackson Laboratories). All in vivo research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council of the National Academies and under the approval of the Institutional Animal Care and Use Committee.



Properties of the ADCs

- Amanitin toxin is non-genotoxic RNA Polymerase II inhibitor licensed from Heidelberg pharma
- Anti-CD117-AM targets HSCs
- Anti-CD45-AM targets HSCs and immune cells

EFFECTIVE TARGET DEPLETION IN VITRO

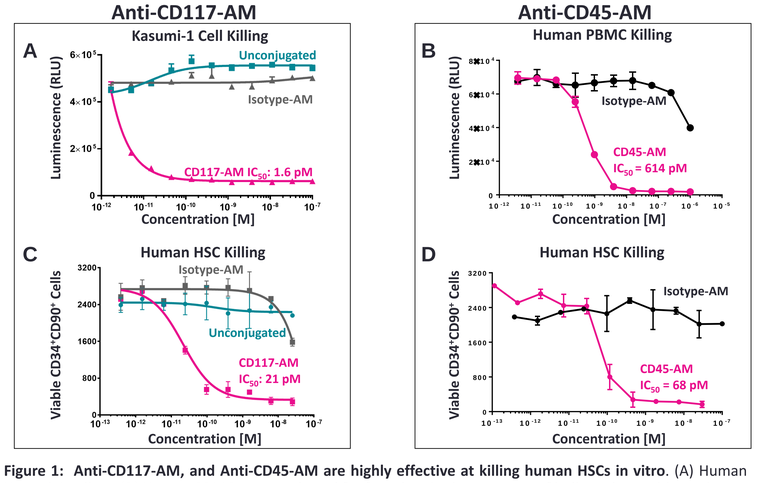
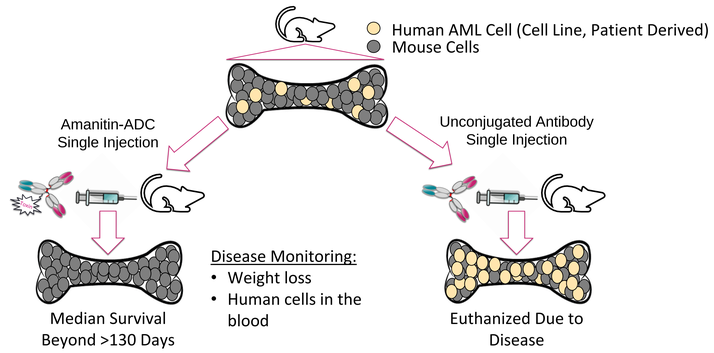


Figure 1: Anti-CD117-AM, and Anti-CD45-AM are highly effective at killing human HSCs in vitro. (A) Human Kasumi-1 cells or (B) Human PBMCs were cultured for four days in the presence of indicated amanitin conjugate or unconjugated Antibody and cell viability was measured by Celltiter Glo. (C,D) Primary human $CD34^+$ bone marrow cells were cultured for 5 days with indicated ADC amanitin conjugate or unconjugated antibody and live CD34+CD90+ HSC counts were determined by flow cytometry.

AML MODEL



ANTI-CD117-AM INCREASES SURVIVAL >3-FOLD IN KASUMI-1 AML XENOGRAFT MODEL

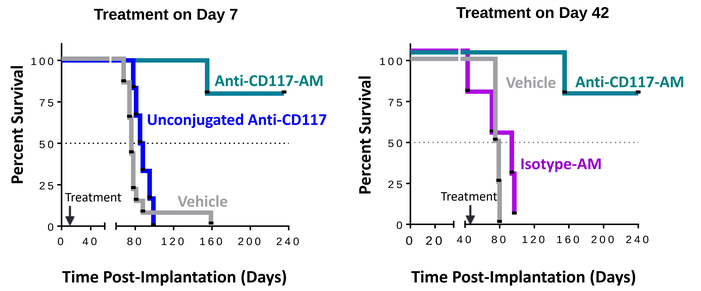


Figure 2: Anti-CD117-AM increases median survival in the Kasumi-1 xenograft model. A single injection of 0.3 mg/kg anti-CD117-AM administered on day 7 or 42 after AML inoculation resulted in a marked increase in survival (median >240 days) compared to vehicle (PBS) treated controls (median 76 days) or unconjugated anti-CD117 antibody (median 86.5 days) (n=6-8 mice/group, p<0.0001).

ANTI-CD45-AM DOUBLES MEDIAN SURVIVAL TO 40 DAYS IN REH-LUCIFERASE ALL XENOGRAFT MODEL

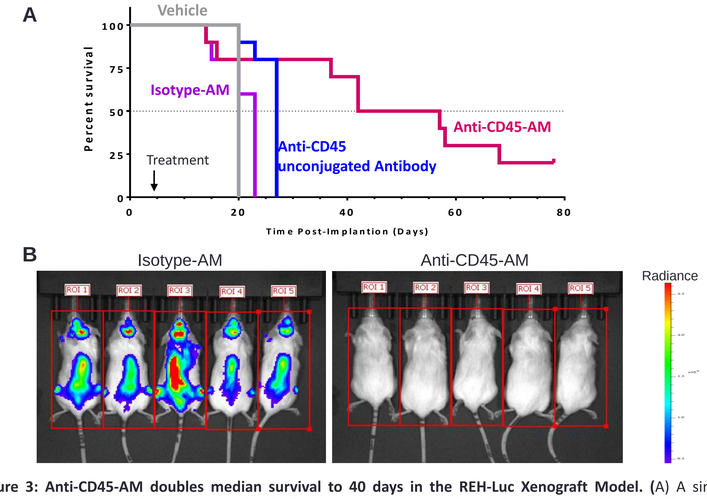
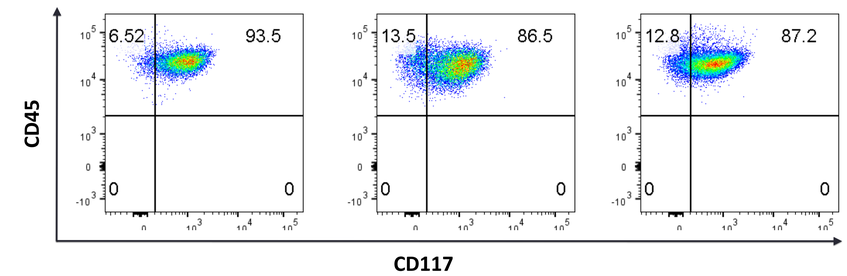


Figure 3: Anti-CD45-AM doubles median survival to 40 days in the REH-Luc Xenograft Model. (A) A single injection of 1 mg/kg anti-CD45-AM on day 5 after ALL inoculation resulted in longer survival by a median of 15 days compared to vehicle (PBS) treated controls or unconjugated anti-CD45 antibody (n=10 mice/group, p<0.0001). (B) Representative bioluminescence signal pseudocolored images were captured using the IVIS imaging system (Perkin Elmer) on day 19 post-implantation of the Anti-CD45-Am and Isotype-AM treatment groups.

ANTI-CD45-AM, AND ANTI-CD117-AM EXTENDS SURVIVAL 2 TO >4-FOLD IN THREE PATIENT DERIVED AML MODELS

A

PDX Model	AML #1	AML #2	AML #3
Type	AML recurrent/Relapse	AML M4/5	AML, M4
Previous treatment	Allogeneic HSCT, Sorafenib, Hydroxyurea, Decitabine	Allogenic HSCT, Induction chemotherapy, consolidation HDACi	No previous treatment reported
Karyotype; Mutation	Normal; FLT3+, NPM1+, DNMT3A+, IDH1+	FLT3 ITD+, FLT3+, TKD+, NPM1+	46, XY, FLT3+, NPM1+



B

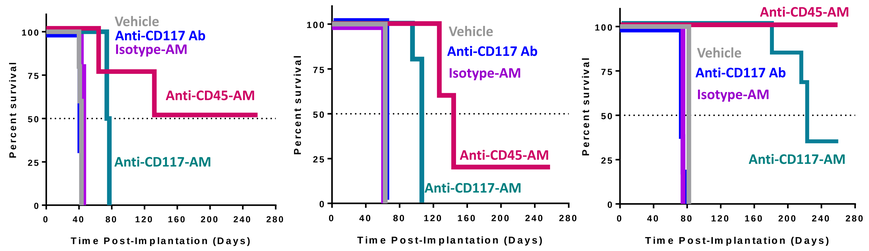


Figure 4: **A single dose of CD45-AM or CD117-AM (1 mg/kg) effectively depletes human leukemic cells in three patient derived AML models.** A) AML PDX model characterization. CD117 and CD45 cell surface expression on CD33+ splenocytes from diseased mice was evaluated by flow cytometry. B) Survival curve of PDX AML mice treated with a single intravenous dose of ADCs (anti-CD45-AM, anti-CD117-AM, ISO-AM), unconjugated anti-CD117 antibody or vehicle (PBS) were administered when 2-5% blasts were observed in the blood (4-5 mice/group/AML-PDX model,). Survival was significantly increased in recipients of anti-CD117-AM, and anti-CD45-AM as compared to vehicle controls.

PDX-AML Model	Median Survival (Days Post Dose, p-value*)		
	AML #1	AML #2	AML #3
Vehicle (PBS)	43	63	82
CD45-AM	195 (p<0.01)	144 (p<0.01)	>280
CD117-AM	75.5 (p<0.01)	106 (p<0.01)	221 (p<0.01)
Isotype-AM	46	63	75
CD117-Naked	43	63	75

Table 1: Median survival (days post dose administration) in PDX AML models and statistical analysis (Log-Rank Test) comparing ADCs against either control group (vehicle [PBS], ISO-AM, and/or unconjugated antibody).

CONCLUSIONS

We have demonstrated that a single dose administration of anti-CD117-AM, or anti-CD45-AM is well tolerated and capable of:

- **Potent killing of human CD34+CD90+ hematopoietic stem cells, AML cell line, and human PBMCs in vitro**
- **Prolonging survival of established leukemia models (cell line and patient derived)**
- **Potent anti-leukemia effects based on these data in humanized murine models with established AML**

Together with prior reports on the potency of anti-CD117-AM and anti-CD45-AM as conditioning agents, these non-genotoxic ADCs may be useful to reduce disease burden in patients with active disease and in recipients of reduced dose conditioning who are at high risk of disease relapse. Next steps are to finalize mAb-linker-toxin construct and select anti-CD117 and anti-CD45 leads for development. Additional work from Magenta's targeted conditioning programs can be viewed in posters 3314, 4526, 3324 and 2041